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SHIVERING AND NON-SHIVERING THERMOGENESIS DURING SUMMIT METABOLISM IN YOUNG LAMBS

BY G. ALEXANDER AND THE LATE D. WILLIAMS

From the C.S.I.R.O., Division of Animal Physiology, The Ian Clunies Ross Animal Research Laboratory, Prospect, New South Wales, Australia

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SUMMARY

1. Summit metabolism of lambs declined steadily from about 3·5 l. O_2/kg.hr during the first day of life, to about 2·0 l. O_2/kg.hr at 2 months of age.

2. The contributions of shivering and non-shivering thermogenesis to these changes were estimated by three independent methods; non-shivering thermogenesis was stimulated by catecholamines in a thermoneutral environment, shivering was suppressed by curariform drugs during summit metabolism, and an attempt was made to suppress non-shivering thermogenesis during summit metabolism by use of the sympatholytic drugs phentolamine and propranolol. Drugs were given by intravenous infusion during measurement of oxygen consumption in a closed circuit respiration chamber.

3. 'Resting' metabolic rate of lambs during the first day of life was increased two to three-fold, from 1 l. O_2/kg.hr, by either adrenaline or noradrenaline infused at 1–10 μg/kg.min. The increase declined with increasing age of lamb and was virtually absent by 3 weeks. The response to catecholamines appeared maximal at the dose levels used.

4. Muscular paralysis induced by suxamethonium or gallamine reduced summit metabolism by about 2 l. O_2/kg.hr in all lambs examined within the first 2 months of life. The residual metabolic rate, and the metabolic response to catecholamines under thermoneutral conditions, declined with age in the same manner, and their magnitudes were similar.

5. Summit metabolism in lambs aged up to 2 months was depressed to varying degrees by the sympathetic inhibitors phentolamine, propranolol and hexamethonium. The depression with propranolol was greater, and the decline with age clearer, than with phentolamine. Hexamethonium and phentolamine depressed blood pressure, propranolol decreased heart rate

and phentolamine and propranolol each suppressed shivering in some experiments.

6. In 1 day-old lambs estimates of non-shivering thermogenesis, by the various methods, ranged from 0·8 to 1·4 l. O_2/kg.hr (mean 1·1 l. or 31 % of summit metabolism), and the estimates of shivering ranged from 1·3 to 1·9 l. O_2/kg.hr (mean 1·6 l. or 46 % of summit metabolism). However, in lambs 1-month old, estimates of non-shivering thermogenesis from sympathetic inhibition (0·6 and 0·8 l. O_2/kg.hr) were considerably higher than estimates from muscular paralysis or stimulation by catecholamines (0·2 and 0·1 l. O_2/kg.hr). It is suggested that the depression of summit metabolism by the sympathetic inhibitors is not solely due to specific inhibition of non-shivering thermogenesis, at least in the older lambs.

7. The possession of a non-shivering thermogenic mechanism in addition to shivering is of clear survival value to new-born lambs.

INTRODUCTION

Most, if not all new-born eutherian mammals, from those born at an early stage of development like the rat (Taylor, 1960) to those born relatively mature like the lamb (Alexander, 1962*a*), respond to cold exposure by increasing oxygen consumption. In most species this increase is mimicked by catecholamine injection (e.g. Moore & Underwood, 1960; Scopes & Tizard, 1963), and there is now convincing evidence that both cold and catecholamines act by stimulating metabolism in brown fat. This is found in quantity in many new-born mammals and its metabolism is under the control of the sympathetic nervous system (Hull & Segall, 1965*a*). The new-born pig and ox appear to lack brown fat and their metabolism is unresponsive to noradrenaline (Le Blanc & Mount, 1967; Jenkinson, Noble & Thompson, 1968).

Shivering, an important source of heat in cold-exposed adults, has usually been dismissed as an insignificant source of heat in the new-born of many species; e.g. dog (Jensen & Ederstrom, 1955), cat (Hill, 1959), rat (Taylor, 1960), man (Brück, 1961) and rabbit (Hull, 1965). From a subjective assessment, Hull (1965) concluded that shivering in the new-born rabbit occurred only when heat production was near maximum, and Brück & Wünnenberg (1965*b*), using electromyography, estimated that shivering supplied only about 10 % of the total heat production in new-born guinea-pigs exposed to air at 8° C.

The present work was undertaken to provide information on the relative contributions of shivering and non-shivering thermogenesis to summit metabolism (Alexander, 1962*a*, *b*) and in the new-born sheep.

To reduce difficulties in the interpretation of the effects of drugs on

metabolism (see Dawes & Mestyán, 1963), three approaches were made. First, summit metabolism was measured before and after blockade of the sympathetic nervous system; secondly, the contribution of shivering was estimated from the reduction in summit metabolism produced by curariform drugs; and, thirdly, catecholamines were infused under thermoneutral conditions in an attempt to provide an independent assessment of the maximum non-shivering contribution.

The results show that shivering is an important source of heat in the newborn lamb exposed to cold.

METHODS

Animals. Fifty Merino lambs from well-nourished ewes were used. Most ewes were brought into an animal house about a week before lambing; the times of birth were known to within 8 hr. The remainder lambed on pasture. Lambs that were examined at several ages remained with their mothers in an animal house during the period of study.

Preparation of lambs. Lambs were removed from their mothers 1–4 hr before the start of the experiment, and wool was clipped from the back to facilitate heat loss. Catheters of polyvinyl chloride (i.d. 0·86 mm, o.d. 1·27 mm, Dural Plastics, Dural, N.S.W.) were then inserted into a jugular vein by the method of Herd & Barger (1964), and into the inferior vena cava via the recurrent tarsal vein through a small skin incision. In some experiments, a polythene catheter (i.d. 0·5 mm, o.d. 0·9 mm) was inserted into the femoral artery or into the anterior metatarsal artery for the measurement of blood pressure; frequently, the end of the catheter lay in the aorta. This catheter was connected to nylon tubing (i.d. 1·0 mm, o.d. 1·34 mm, 'Portex', Boots) at skin level. Outside the skin, catheters were jacketed in electrically heated plastic tubing to prevent freezing.

When curariform drugs were to be used, a rubber tracheotomy tube (i.d. 7–8 mm, o.d. 10–11 mm) with a bevelled end was inserted into the trachea through a neck incision and was sewn to the skin. All operations were done under local anaesthesia.

Electrodes of nickel-plated brass safety-pins, 2·4 cm long, were fastened to the skin for electromyographic and electrocardiographic recording; a thermocouple was inserted 5 cm into the rectum and others were fastened with waterproof cement (Selley) to the ear, back, tail, fore- and hind-legs, just above the hoof and over the proximal end of the metatarsus.

Catheters, electrode and thermocouple leads were secured to a tape harness and the lamb was placed in a wire cage, 59 × 30 × 57 cm high. The animal was allowed to stand, but free movement of the limbs was limited to about 10 cm in any direction by tapes attaching the legs to the cage. A sling of cord netting (4 cm mesh), suspended just below the trunk, provided support when the lamb relaxed or was paralysed.

Finally, to facilitate heat loss from the larger lambs, water was poured over them and rubbed into the coat.

Oxygen consumption was measured in an improved version of the closed circuit respiration apparatus described previously (Alexander, 1961*a*). The animal chamber (68·5 × 37 × 68·5 cm) and wind ducts were totally immersed in 40 % (v/v) ethylene glycol in water and the lid was sealed by a rubber gasket and clamps. Wind speeds of up to 34 km/hr could be obtained through a fan of continuously variable speed, manually controlled, and the air in the chamber could be controlled at temperatures down to −15° C. Water and carbon dioxide were removed in an absorption train of increased capacity (maximum flow 160 l./min). Dried calcium chloride was used, as before, to absorb water, and carbon dioxide was removed by soda asbestos (Carbosorb, B.D.H., 6–12 mesh) mixed with twice its volume of Perspex shavings to maintain porosity. The larger (2 l.) absorption cylinders were weighed on a highly damped balance of 4 kg capacity (Sauter). Oxygen was admitted from a spirometer, as described previously, and changes in oxygen partial pressure in the chamber were deter-

mined by a paramagnetic oxygen meter (Beckman, E 2) through which a stream of dried chamber air was drawn. Samples of chamber air were withdrawn into a 10 ml. hypodermic syringe, and analysed for CO_2 and methane using an 0·5 ml. Scholander gas analyser (Scholander, 1947); a heater element in the recessed plunger permitted combustion of the sample for conversion of methane to CO_2.

The volume of oxygen, at s.t.p., consumed by the subject was calculated as reported previously (Alexander, 1961*a*); the volume delivered from the spirometer was first corrected for changes in the composition of the 260 l. of air in the apparatus, for errors in the measurement of dry gas pressure and temperature, and for small unidirectional leaks in the apparatus. Oxygen consumption was usually determined at intervals of 10–20 min throughout an experiment by making appropriate readings and analyses. Carbon dioxide production, measured by weighing absorption cylinders and correcting for changes in the CO_2 content of the chamber, was usually determined over longer intervals, owing to the necessity of interrupting an experiment to weigh cylinders.

Lambs treated with curariform drugs were forced ventilated by a respiration pump (Palmer 'Ideal'). Air was drawn from the chamber by the pump and delivered to the lungs via one arm of a T-piece attached to the tracheotomy tube; air left the lungs under elastic recoil via the other arm and was vented, via the pump, to the air stream flowing from the chamber to the absorption train. Ventilation rate was 18 or 32/min and the stroke volume was adjusted to provide a normally appearing expansion of the chest wall (50–200 ml. depending on the size of the lamb).

Body temperatures were recorded with copper-constantan thermocouples connected to a Speedomax recorder (Leeds and Northrup, type G).

Blood pressure was measured by a Statham strain gauge transducer (Beckman, type P 23 Db) connected to a physiological recorder ('Offner Dynograph', Beckman). The arterial cannula was filled with ethylenediaminetetra-acetate (EDTA, 2·5 g/100 ml. NaCl solution (0·9 g/100 ml.)) and small amounts were flushed through from time to time to maintain patency. Blood pressure was not measured in the early phases of the investigation, and not always subsequently, partly because of difficulties in repeated catherization and sometimes because catheters became occluded in the animal, particularly when inserted in the metatarsal artery.

Heart rate was indicated on the blood pressure trace, but was usually monitored by electrocardiography using the same recorder. Electrodes were placed on the back (earth) and right front shoulder and left thigh.

Shivering. A semi-quantitative assessment of the intensity of shivering was obtained by electromyography during most of the study. The three safety-pin electrodes were attached to the skin, about 4 cm apart, over the thigh muscles of one leg and connected to the recorder through an integrating coupler.

Drug infusion. Drugs in pyrogen-free sterile NaCl solution (0·9 g/100 ml.) were infused into the jugular vein by a roller-type infusion pump at the rate of 0·1–0·4 ml. fluid/min, depending on dose and concentration of drugs; during control periods, saline was given alone. Dose rate was adjusted according to the weight of the lamb.

Stimulation of brown fat metabolism was attempted with the bitartrate salts of adrenaline and noradrenaline (British Drug Houses Ltd.) freshly dissolved in saline with ascorbic acid (0·3 g/100 ml.) as preservative. Doses of catecholamines are expressed as base.

Inhibition of brown fat metabolism was attempted with the sympathetic α-receptor inhibitors phentolamine hydrochloride ('Regitine', Ciba) and phenoxybenzamine hydrochloride ('Dibenzyline', Smith, Kline and French), with the β-receptor inhibitor propranolol ('Inderal', I.C.I.) and with the ganglion blocking agent hexamethonium bromide ('Vegolysin', May and Baker).

Paralysis of voluntary muscles was initially induced with gallamine triethiodide ('Flexedil', May and Baker) but, as treated animals failed to recover, suxamethonium chloride 'Anectine', Burroughs Wellcome) was used for most of the experiments.

Administration of drugs. Doses required to produce sympathetic blockade were examined in preliminary experiments where several doses, each 2–3 times higher than the preceding one, were used. Blockade was indicated by changes in heart rate, blood pressure, skin temperature or summit metabolism. Blockade was judged to be complete when increased doses produced no further response.

Before a test period priming doses of sympatholytic drugs were routinely given as single injections or by infusion for 15 min. Infusions continued during one or all of several consecutive test periods at the rate of $\frac{1}{15}$th of the priming dose per minute. Doses stated in the text refer to the priming dose.

Catecholamines were given by infusion following a priming dose equal to that given during 3–5 min of infusion, and the doses stated in the text refer to the infusion rates. Doses required to produce maximal thermogenic stimulation were examined by increasing the infusion rate between two consecutive measurements of metabolic rate.

Blood sampling. Catheters for blood sampling were filled with EDTA (2·5 g/100 ml.) in sterile physiological saline (0·9 g NaCl/100 ml.), and samples of 2 or 7 ml. were withdrawn at intervals during the experiments (see Alexander, Mills & Scott, 1968). The catheter was refilled with EDTA solution after sampling. Usually, between 20 and 30 ml. blood was removed during the experiment ($< 15\%$ of estimated blood volume).

Experimental procedure. Summit metabolism was measured as previously described (Alexander, 1962*a*). The cage containing the lamb was placed in the chamber, usually at -5 to $-15°$ C air temperature. Cannulae were led through a rubber stopper compressed by a retaining nut to provide a seal, the electrical connexions were made, the lid was closed and the chamber was ventilated with room air. The fan was started and wind speed adjusted until rectal temperature began to fall. The system was then closed to atmosphere by connecting the absorption train, and the spirometer was turned on. The wind speed was adjusted from time to time to maintain a clear fall in rectal temperature (about 0·5 to 1° C/15–20 min). Summit oxygen consumption was measured over 2–3 'control' periods of 10–20 min each; infusion of drugs then commenced, and the measurements were repeated during 2 or 3 'test' periods. Means of the replicate determinations were used in the analyses of results. To avoid excessively low rectal temperatures the wind was usually turned off for up to 1 hr between control and test periods, the chamber opened, and the lamb re-warmed by a stream of heated air blown in from above. Where the administration of drugs was expected to reduce summit metabolism markedly, chamber temperature was elevated by up to 20° C after the control periods so that the fall in rectal temperature could be controlled during measurement of the reduced summit metabolism. It was usually possible to select an appropriate chamber temperature and avoid trial and error adjustments. When rectal temperature had returned to normal, the chamber was closed again, drug infusion was started and, after 5–10 min, summit metabolism was measured as before. Where a further reduction in summit metabolism was to be induced, the same procedure was repeated and chamber temperature was usually elevated to about 20° C.

Muscular paralysis was induced while the lid was open; the animal was connected to the respiration pump when breathing became shallow and irregular.

For measurement of metabolism unstimulated by cold ('basal' metabolism), lambs were placed in the chamber at temperatures ranging from 24 to 30° C, depending upon age and coat length. These temperatures were near the lower end of the thermo-neutral region: at higher temperatures, the thermogenesis induced by catecholamines could have resulted in an excessive rise in rectal temperature. In each experiment control measurements were first made over 2 or 3 periods of 45–60 min. During catecholamine infusion, measurements were restricted to $\frac{1}{2}$ hr to reduce the effects of increased rectal temperature and activity. Further control measurements were usually made after infusion had been discontinued and rectal temperature had been allowed to return to normal. Usually another catecholamine was then given, and the effects on summit metabolism examined as before.

Recovery treatment. After the experiments artificial respiration of paralysed animals was

continued until strong respiration was re-established. Catheters, electrodes and tracheotomy tubes were removed, wounds were treated with antibiotic ointment and sutured, and the lamb was injected intramuscularly, with penicillin (100,000 i.u.) and streptomycin (0·25 g) before being returned to its mother. Lambs sucked readily soon after return to their mothers and growth between experiments was normal (about 0·2 kg/day—Figs. 3–5). The clipped area of skin was covered with a felt coat between experiments.

RESULTS

Results from two series of experiments are presented but not distinguished. In a preliminary series, lambs were usually used only once, but in the main study most lambs were examined at several ages, from less than 24 hr to about 8 weeks. There was no evidence, from comparison of the two series, that responses of lambs were affected by previous treatments.

Control experiments. Since treatments were applied after control measurements of summit metabolism, treatment and duration of cold exposure

TABLE 1. Effect of experimental procedure on summit metabolism

Age of lamb (days)	Summit metabolism (l. O_2/kg.hr) Control periods 1	2	3	Sham test periods 1	2	3	Difference between means (control − test)
0·3	4·1	4·1	—	3·5	3·9	—	0·40
1	4·2	4·0	—	3·6	3·9	—	0·35
2	2·7	2·9	—	2·2	1·8	—	0·80
2	3·6	4·0	—	3·8	3·9	—	−0·05
3	4·0	3·9	—	4·1	3·9	—	−0·05
5	4·0	4·1	3·6	4·2	3·7	4·1	−0·10
5	2·7	2·7	—	2·4	2·7	—	0·15
8	3·5	3·6	—	3·1	3·5	—	0·25
12	3·8	3·8	—	3·3	3·5	—	0·40
s.d.* of replicate determinations	0·17			0·23			
Means	3·63			3·38			0·24 (s.d. = ±0·29) (0·05 > P > 0·02)

* Calculated from pooled error variance from each set of determinations.

were confounded. Nine lambs, aged 8 hr to 12 days, were therefore examined by the normal experimental routine; saline was infused but no drugs were given during the 'sham' test period.

Summit metabolism in six of the nine lambs was lower during the test period than during the control period (Table 1; Fig. 1) and the mean fall of 7 % of the control value was significantly greater than zero. There was no obvious relationship between age and fall in summit metabolism.

R.Q. tended to be lower during the test period than the control period (Table 2) but the mean difference was not significant.

In each experiment mean blood pressure and heart rate were slightly lower during the test period than during the control period (Table 3). Usually heart rate and sometimes blood pressure tended to increase fairly rapidly upon the first exposure to cold, and then to fall slowly (Fig. 1).

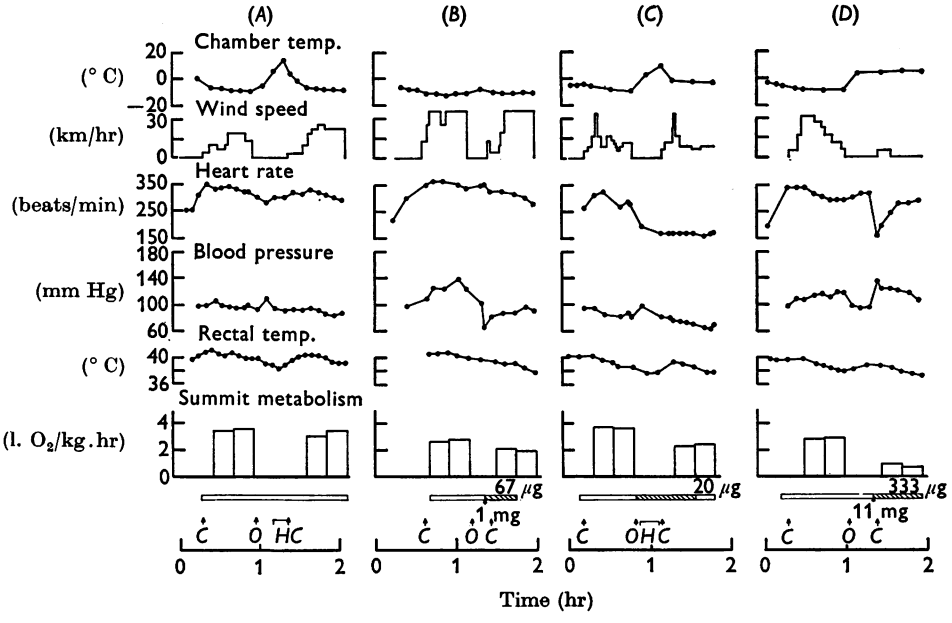

Fig. 1. Effect of drugs on summit metabolism: typical experiments. (*A*) Control experiment: lamb (3·5 kg, 8 days old) was subjected to experimental routine without infusion of drugs. (*B*) Effect of phentolamine: lamb no. 46, 7·6 kg, 18 days old. (*C*) Effect of propranolol: lamb no. 227, 7·5 kg, 31 days old. (*D*) Effect of suxamethonium: lamb no. 290, 3·7 kg, < 12 hr old. Lamb numbers are given to permit cross-reference to data presented by Alexander, Mills & Scott (1968). The time scale begins where the lamb was placed in the chamber. Procedures: *C*, chamber closed; *O*, chamber opened; *H*, external heat applied. □, saline infusion; ▨, drug infusion. Infusion rates (μg/kg.min) are shown above the bar; priming doses (mg/kg) are indicated below the bar.

TABLE 2. Effect of experimental treatments on respiratory quotient

Treatment	No. of comparisons	Mean respiratory quotient ± S.E.M. Control period	Test period	Significance of mean difference
Summit conditions				
No drugs (sham experiments)	6	0·87 ± 0·03	0·82 ± 0·04	N.S.
Phentolamine	9	0·91 ± 0·03	0·85 ± 0·02	N.S.
Propranolol	15	0·90 ± 0·02	0·84 ± 0·02	$P = 0·05$
Suxamethonium	17	0·89 ± 0·02	0·69*	*
Basal conditions				
Catecholamines	7	0·82 ± 0·04	0·87 ± 0·04	N.S.

N.S. = Not significant.

* S.E. and significance not calculated because test R.Q.s probably affected by hypo- or hyperventilation.

TABLE 3. Effect of experimental treatment on heart rate and blood pressure

Treatment	Heart rate (beats/min)			Blood pressure (mm Hg)		
	No. of comparisons	Mean during control period	Mean change after drug ± S.E.M.	No. of comparisons	Mean during control period	Mean change after drug ± S.E.M.
Summit conditions						
No drugs (sham experiments)	9	309	−18 ± 8	6	85	−5 ± 1
Phentolamine	14	320	−24 ± 4 N.S.	9	96	−29 ± 2***
Propranolol	18	318	−137 ± 5***	11	110	−11 ± 2 N.S.
Suxamethonium	17	309	−74 ± 5***	12	100	+10 ± 3***
Basal conditions						
Noradrenaline	12	197	+5 ± 18 N.S.	7	92	+41 ± 7***
Adrenaline	11	229	−37 ± 18 N.S.	7	90	+46 ± 8***

Significance of differences from 'sham' values in 'Summit' experiments, or from zero in 'Basal' experiments.
N.S. = Not significant.
*** = $P \leqslant 0{\cdot}001$.

TABLE 4. Effect of variations in dose and methods of administration of drugs on metabolic rate

Treatment	Effect of increasing dose on mean oxygen consumption (l. O_2/kg.hr)					Effect of stopping infusion on mean oxygen consumption (l. O_2/kg.hr)				
	No. of comparisons	Control period	After low dose*		After continued infusion often at higher rate	No. of comparisons	Control period	During* infusion		After infusion
Summit conditions										
No drugs (sham experiments)	9	3·7	3·4	N.S.	3·	—	—	—		—
Phentolamine	11	3·2	2·5 (0·5 mg/kg)	N.S.	2·4 (†)	7	3·1	2·2 (1 mg/kg)	N.S.	2·5
Propranolol	7	3·5	2·4 (75 μg/kg)	N.S.	2·3 (†)	13	2·9	1·9 (0·3 mg/kg)	N.S.	2·1
Suxamethonium	20	3·0	1·1 (300 μg/kg.min)	N.S.	1·1 (300 μg/kg.min)	—	—	—		—
Basal conditions										
Catecholamines§	7	0·9	1·5 (3 μg/kg.min)	N.S.	1·7 (9 μg/kg.min)	—	—	—		—

N.S. Mean difference not significant.

* Mean doses are shown in brackets (see Methods).

† Accumulative dose at least 3× initial priming dose.

§ Experiment excluded where 1 μg noradrenaline was followed by 5 μg which greatly increased metabolic rate.

Blockage with α-sympatholytic agents. Blockade with phentolamine was indicated by a rise in ear temperature, a fall in blood pressure, and a reduction in summit metabolism; there was no clear effect on heart rate (Fig. 1; Table 3). Thermogenic blockade appeared complete after 100 μg/kg but marked changes in skin temperature were not always seen after doses

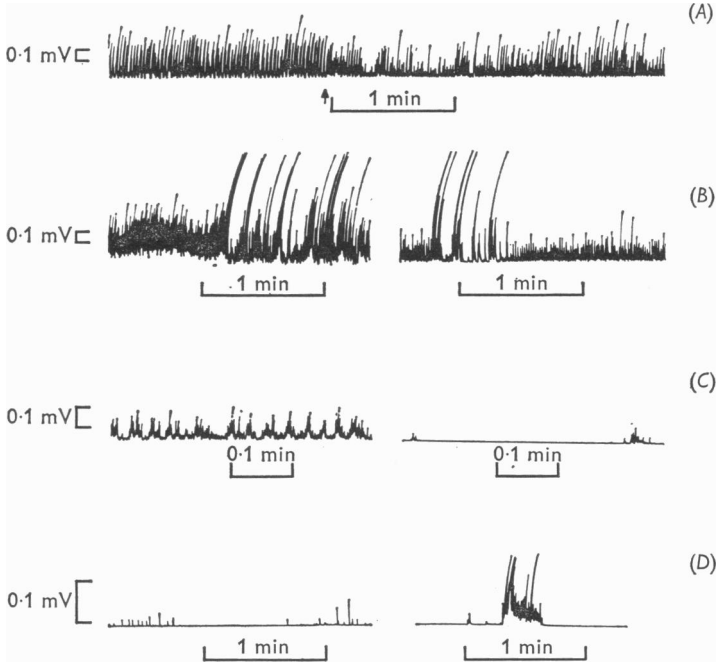

Fig. 2. Electromyograms of lambs under various treatments. The time and voltage scale vary from trace to trace. (*A*) Summit metabolism and phentolamine (lamb 5 days old). Single I.V. injection (↑) of 10 μg/kg resulted in transient reduction in the intensity of shivering. (*B*) Summit metabolism and propranolol (lamb < 12 hr old). Before (left) and after (right) infusion of 20 μg/kg.min for 40 min. The intensity of shivering was markedly reduced. *Note*: Transient suppression of shivering after voluntary movement represented by largest discharges. (*C*) Summit metabolism and suxamethonium (lamb 7 days old). Before, and 15 min after I.V. injection of 3 mg/kg followed by infusion at rate of 0·17 mg/kg.min. Complete suppression of activity of voluntary muscle was not attained at this rate. (*D*) Resting metabolism in thermo-neutral environment (28° C) with saline infusion only (lamb 17 days old); examples of activity. The smaller discharges represent very slight voluntary movements; the large series of discharges was associated with the lamb bleating and moving restlessly.

below 600 μg/kg. A priming dose of 1 mg/kg was therefore used routinely. A typical experiment is illustrated in Fig. 1. After priming doses ranging from 0·1 to 1 mg/kg continued infusion, often at increasing rates, produced no further reduction in summit metabolism (Table 4).

In some experiments shivering was temporarily suppressed (Fig. 2) immediately after administration of the priming dose, and there appeared to be a slight, though not significant recovery in summit metabolism after the infusion of phentolamine ceased (Table 4). This effect was more variable than in any other homogeneous group of measurements (Table 5), and it appears that phentolamine may exert some short-term adverse effect on summit metabolism, particularly in some individuals, in addition to a longer term sympathetic blockade.

TABLE 5. Variability of replicate measurements of summit metabolism during 'sympathetic receptor blockade'

Treatment		Degrees of freedom	Standard deviation
Infusion of 'α'-blocker throughout test period	Control	12	0·27
	Test	14	0·27
Infusion of 'α'-blocker during first test period only	Control	7	0·26
	Test	13	0·45
Infusion of 'β'-blocker throughout test period	Control	10	0·15
	Test	14	0·23
Infusion of 'β'-blocker during first test period only	Control	14	0·29
	Test	14	0·19
Bartlett's χ^2 test for homogeneity of variances		Corrected χ^2 = 17·90 with 7 degrees of freedom $0{\cdot}02 > P > 0{\cdot}01$	

Adequate blocking doses were given in twenty experiments involving thirteen lambs, aged from a few hours to 78 days; phentolamine reduced summit metabolism in each experiment (Fig. 3); the mean fall $\pm$ S.D. was $0{\cdot}72 \pm 0{\cdot}41$ l. O_2/kg.hr. Summit metabolism declined significantly with increasing age and the reduction tended to be greatest at the lowest ages; this trend approached significance (Table 6). There was also a significant decline in residual summit metabolism with advancing age (Table 6).

R.Q. was usually lower after phentolamine than during control measurements, but the difference was not significant (Table 2).

The α-blocking agent phenoxybenzamine was also used in two experiments (lambs 13 and 23 days old). Priming doses of 8 mg/kg resulted in a considerably greater fall in summit metabolism (from 5·0 to 3·2, and from 2·4 to 1·2 l. O_2/kg.hr, Fig. 3) than induced by phenotolamine (mean falls 0·72 and 1·50 l. O_2/kg.hr, $t = 2{\cdot}51$, degrees of freedom (D.F.) = 20, $P = 0{\cdot}02$).

Blockade with a β-sympatholytic agent (propranolol). Achievement of blockade with propranolol was indicated by a considerable fall in heart rate and a reduction in summit metabolism (Fig. 1, Table 3); there were no observable changes in skin temperature or blood pressure. Blockade appeared complete after a priming dose of 75 μg/kg, but a dose of 300 μg/kg was adopted for routine use. Doses above 75 μg/kg produced no further

reduction in summit metabolism (Table 4). Results from a typical experiment are shown in Fig. 1.

As with phentolamine propranolol clearly reduced the intensity of shivering in some experiments particularly as the experiment progressed (Fig. 2). However, there was no marked or rapid increase in summit metabolism after infusion ceased (Table 4).

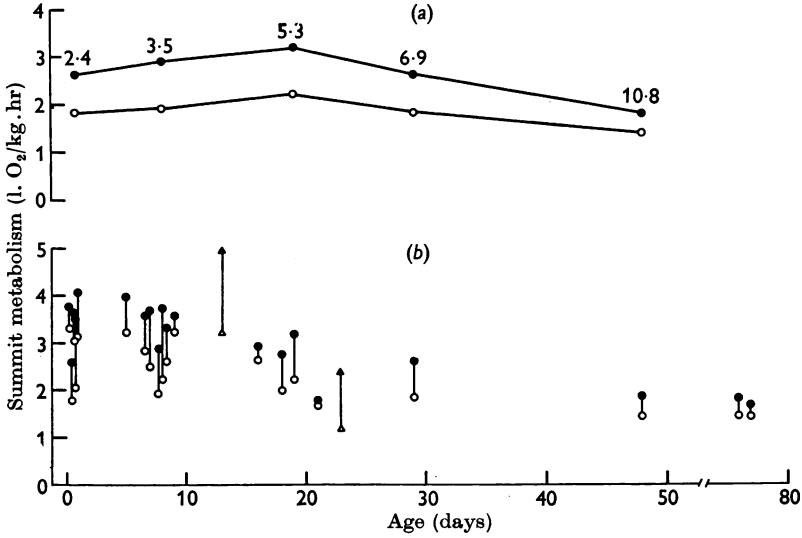

Fig. 3. Depression of summit metabolism by two α-sympatholytic drugs (for doses see text), in relation to age. (*a*) Results in one lamb 12 hr to 48 days old (no. 197). The weight in kg of the lamb at each age examined is shown on the graph. (*b*) Results from all lambs. ●, Before treatment with phentolamine; ○, after treatment with phentolamine; ▲, before treatment with phenoxybenzamine; △, after treatment with phenoxybenzamine.

Adequate blocking doses were given in twenty-one experiments on twelve lambs aged from 12 hr to 50 days (Fig. 4). Propranolol reduced summit metabolism in each experiment (mean reduction ± S.D. = 1·17 ± 0·55 l. O_2/kg.hr). Summit metabolism during the control period declined with increasing age as in the previous section and the reduction tended to fall with advancing age (Table 6). The mean reduction was greater after propranolol than phentolamine (t = 2·95, D.F. = 39, 0·01 > P > 0·001). There was no apparent relation between residual summit metabolism and age (Table 6).

The mean R.Q. after propranolol blockade was significantly below the mean of corresponding control periods (Table 2).

Ganglionic blockade. Ganglionic blockade after injection of 5 mg/kg of hexamethonium was indicated by a rise in temperature of the extremities,

TABLE 6. Relation of oxygen consumption to age, before and after treatment with various drugs

Treatment	No. of experiments	The correlation coefficient (r). regression coefficient (b) and their significance: Before treatment r	Before treatment b*	Before treatment P	After treatment r	After treatment b*	After treatment P	Difference r	Difference b*	Difference P
Summit conditions										
Phentolamine	20	−0·77	−0·027	0·001 > P	−0·68	−0·019	0·001	−0·40	−0·001	0·1 > P > 0·05
Propranolol	21	−0·53	−0·027	0·02 > P > 0·01	−0·29	−0·009	N.S.	−0·41	−0·002	0·1 > P > 0·05
Suxamethonium	18	−0·70	−0·026	0·001	−0·78	−0·028	0·001 > P	+0·07	—	N.S.
Sympathetic block plus paralysis	8	—	—	—	−0·39	−0·008	N.S.	—	—	—
Basal conditions										
No drugs	12	−0·88	−0·009	0·001 > P	—	—	—	—	—	—

* l. O_2/kg.hr.day.

and a fall in summit metabolism. Blood pressure fell markedly by about 40 mm Hg in the one experiment in which it was monitored. Thermogenic blockade appeared complete, as continued infusion produced no further fall in summit metabolism.

The mean reduction ± S.D. in summit metabolism in the four lambs examined (aged 2–19 days) was 1·30 ± 0·34 l. O_2/kg.hr, which was significantly higher than that after phentolamine (t = 2·63, D.F. = 22, 0·20 > P > 0·05), and of the same order as that after propranolol.

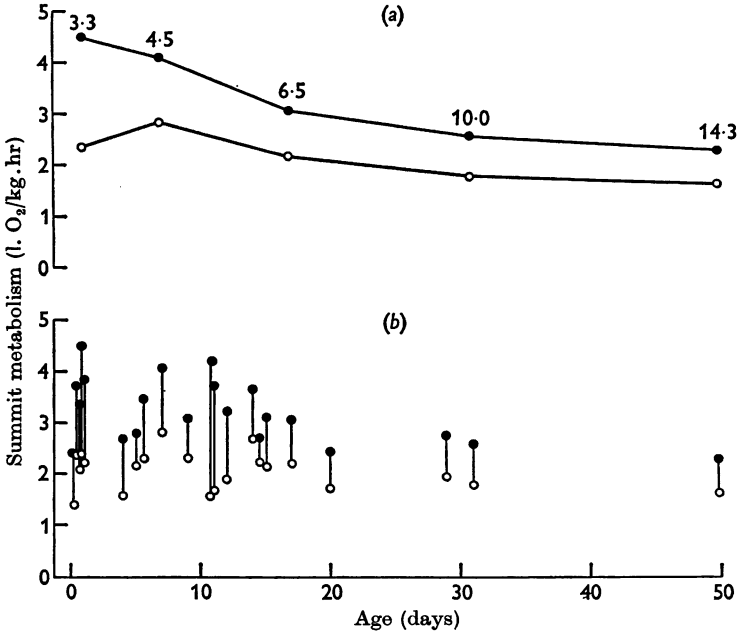

Fig. 4. Depression of summit metabolism induced by propranolol (for doses, see text), related to age of lamb. (*a*) Results in one lamb 1–50 days old (no. 194). The weight in kg of the lamb at each age examined is shown on the graph. (*b*) Results from all lambs examined. ●, before propranolol; ○, after propranolol.

Noradrenaline, infused in doses of 3·3–13·3 μg/kg.min within an hour of ganglionic blockade in three of the animals, raised summit metabolism by 0·8, 0·9 and 0·5 l. O_2/kg.hr.

Paralysis of voluntary muscles. Doses from 5 to 13 μg/kg of suxamethonium chloride given over several minutes were required to induce paralysis. Paralysis appeared incompletely maintained by infusion of 150 μg/kg.min (two experiments), as indicated by occasional spikes on the myogram (Fig. 2), but there was no shivering. With 300 μg/kg.min there was no indication of muscular activity throughout the experiments, and this infusion rate was used routinely.

Heart rate usually fell markedly during the administration of the paralysing dose, but tended to return towards normal as the infusion continued. There were also marked irregularities in the electrocardiogram during induction of paralysis. Blood pressure was usually slightly elevated but tended to return towards normal during infusion (Fig. 1, Table 3). Suxamethonium chloride produced no obvious changes in skin tem-

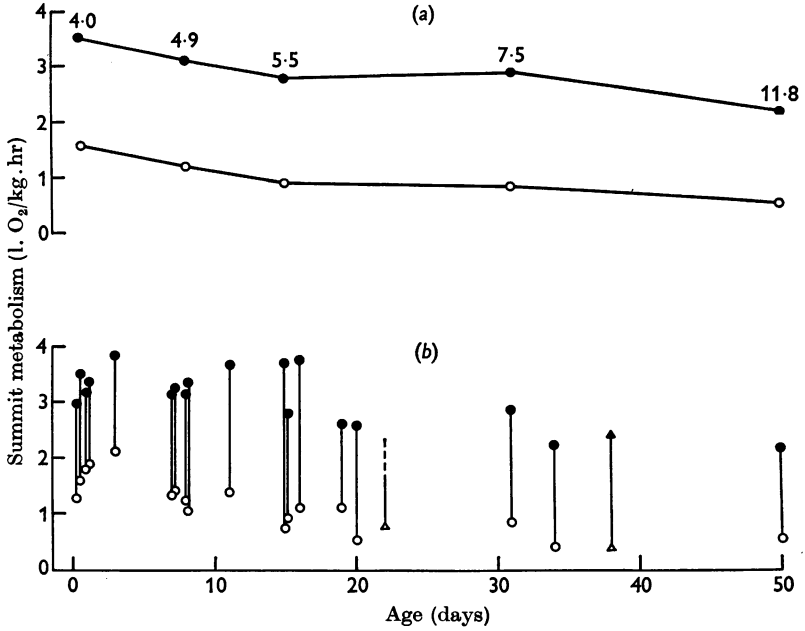

Fig. 5. Depression of summit metabolism, due to muscular paralysis induced by suxamethonium chloride or gallamine (for doses, see text), related to age of lamb. (*a*) Results in one lamb, from less than 1 day to 50 days old (no. 290). The weight in kg of the lamb at each age examined is shown on the graph. (*b*) All lambs examined. ●, before suxamethonium; ○, after suxamethonium; ▲, before gallamine; △, after gallamine. A control measurement in one gallamine-treated lamb was not made.

perature. Although blood P_{CO_2} and P_{O_2} were not monitored, adequacy of ventilation was indicated in most experiments by the bright red colour of venous blood samples; venous P_{O_2} was obviously higher after paralysis than before.

Summit metabolism fell markedly after paralysis in each of the eighteen experiments on normal lambs (eight lambs aged from 6 hr to 50 days), and continued infusion did not produce a progressive decline (Table 4). Summit metabolism, both during the control period and after paralysis, again declined with age, but the decline after suxamethonium was unrelated to age (Fig. 5; Table 6). The mean decline ± S.D. was 1·93 ± 0·41 l.

O_2/kg.hr. Suxamethonium reduced the R.Q. in most experiments (Table 2), but eight of seventeen values obtained after paralysis were below the R.Q. for pure fat. This could have resulted from hypercapnia due to underventilation in some experiments, but the consistency of the results (Fig. 5; Table 6) indicate that the depression in summit metabolism was little affected by variations in ventilation.

Gallamine triethiode was also used in two experiments (Fig. 5) with similar results to those produced by suxamethonium. However, both gallamine-treated lambs failed to recover. After suxamethonium, recovery

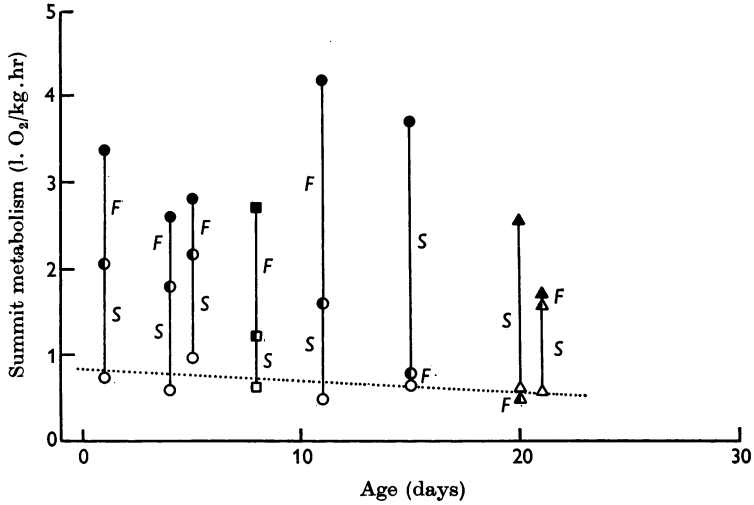

Fig. 6. Effect of simultaneous sympathetic blockade and muscular paralysis on summit metabolism in lambs of different ages.

	Propranolol	Phentol-amine	Hexa-methonium
Control	●	▲	■
After 1st treatment	◐	◭	◧
After both treatments	○	△	□

Depression due to sympatholytic drug indicated by '*F*', depression due to muscular paralysis indicated by '*S*'. For doses of drugs, see text.

was usually rapid, provided artificial respiration was continued until spontaneous respiration became strong; most lambs survived the treatment.

Combined effects of sympathetic blockade and muscular paralysis. Phentolamine, propranolol or hexamethonium were given to eight lambs (1–21 days old), either before or after the voluntary muscles had been paralysed with suxamethonium chloride. In agreement with the results already presented, sympathetic blockade produced almost no effect on summit

metabolism in the three lambs older than 2 weeks (Fig. 6), whereas muscular paralysis reduced summit metabolism substantially in all lambs.

The metabolic rate remaining after sympathetic blockade and muscular paralysis appeared to fall slowly with increasing age (Table 6).

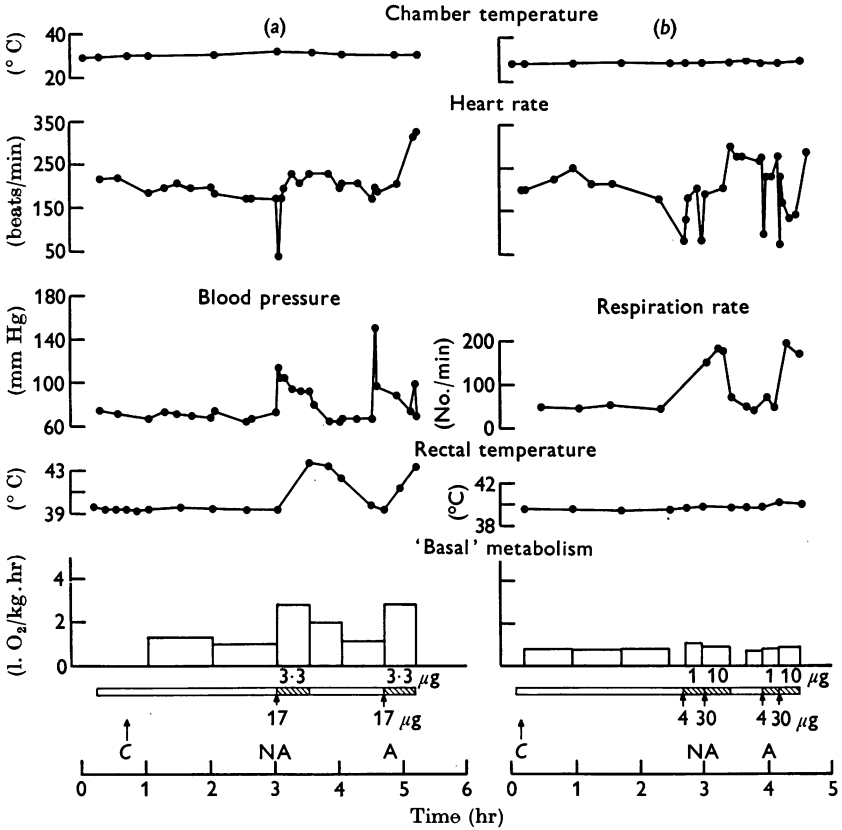

Fig. 7. Effect of catecholamines on 'basal' metabolism and other parameters in two experiments on the same lamb (no. 195). (*a*) 3·9 kg, 12 hr old. (*b*) 6·9 kg, 17 days old. See legend of Fig. 1 for conventions and key to symbols. Noradrenaline (NA) was given first; then adrenaline (A).

Effect of catecholamines on metabolism under thermo-neutral conditions ('basal' metabolism). The effect of catecholamines on basal metabolism was examined in twelve experiments on three lambs, each used at four ages ranging from 12 hr to 45 days. From the control measurements and other experiments (unpublished) the standard deviation of measurement of basal metabolism was calculated to be 0·11 l. O_2/kg.hr. In most experiments, lambs remained quiet during control periods, although there were

occasional bursts of muscular activity (Fig. 2). It was not possible with the present technique to exclude short periods of activity from the periods of measurement.

Both adrenaline and noradrenaline were examined in eleven of the twelve experiments; adrenaline was given first in one lamb and noradrenaline in two. Typical experiments are shown in Fig. 7.

Infusion rates of 10 μg/kg.min were used routinely, but there was little evidence that 10 μg was more effective than 1 or 5 μg. On the eight

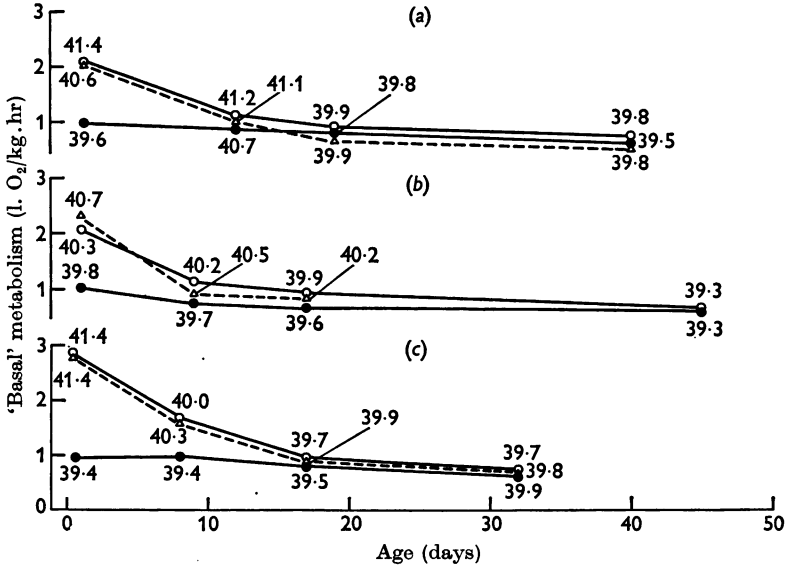

Fig. 8. Elevation in 'basal' metabolism in early life, induced by infusion of catecholamines (for doses, see text). Results from the three lambs examined are shown. (*a*) Lamb 196—adrenaline then noradrenaline. (*b*) Lamb 291—noradrenaline then adrenaline. (*c*) Lamb 195—noradrenaline then adrenaline. ●, control metabolic rate. High metabolic rates associated with excessive movement during control periods have been excluded. ○, metabolic rate during infusion of noradrenaline. △, metabolic rate during infusion of adrenaline. The results are from the first measurement period after start of infusion, except in the first experiment on lamb 196 where 1 μg/kg.min of noradrenaline produced little effect; here, the mean response after 1 and 5 μg/kg.min is plotted. Mean rectal temperatures during periods of measurement of metabolic rate are shown on the figure.

occasions (Table 4) when increasing doses were given during two consecutive periods, the increased response to the higher dose was, with one exception (1 μg followed by 5 μg), no greater than could be expected from the elevated rectal temperature.

Both catecholamines increased basal metabolism two- to threefold in the three lambs when less than 2 days old (Fig. 8); the mean increase

$\pm$ S.E.M. of $1{\cdot}39 \pm 0{\cdot}24$ l. O_2/kg.hr was significantly greater than zero ($P < 0{\cdot}05$, with only 2 degrees of freedom), the increase declined with age and by 3 weeks there was very little or no effect of catecholamine infusion.

Marked increases in metabolic rate and rectal temperature were accompanied by increases in respiration rate. However, in some experiments on the older lambs, where rectal temperature changed little and increases in metabolic rate were equivocal, respiration rate clearly increased (Fig. 7) suggesting a specific effect of infusion on respiration.

Blood pressure increased markedly, and sometimes doubled, during catecholamine infusion; heart rate was usually depressed temporarily after the priming dose, but the mean response was very variable (Fig. 7; Table 3).

The respiratory quotient tended to increase after catecholamine infusion (Table 2).

DISCUSSION

The study of maximum metabolic rates, attained during cold exposure, is hampered by the instability of animals under conditions of maximum heat production; rectal temperature must be induced to fall to indicate that summit metabolism is achieved, and summit metabolism cannot be maintained indefinitely (Alexander, 1962*a*) to allow the imposition of various treatments. Thus there is a bias towards reduction in summit metabolism during any treatment applied after a control period. However, control experiments, the consistency of replicate measurements (coefficient of variation about 10%) and earlier experiments (Alexander 1962*a*) in which lambs were subjected to cold continuously for several hours, indicate that any fall of more than 0·4 l./kg.hr consistently produced by a treatment was almost certainly due to the treatment.

Interpretation of the data is rendered far more difficult by uncertainties about 'side effects' and efficacy of the pharmacological agents used than by uncertainties of method. Indeed, it would be surprising if such an intense physiological effort as summit metabolism were not adversely affected by alterations in blood flow to the various thermoregulatory tissues during sympathetic blockade and treatment with suxamethonium. Even the criterion of Dawes & Mestyán (1963) that blood pressure was not affected cannot be regarded as adequate. In the present experiments, phentolamine and hexamethonium, produced marked reductions in blood pressure (Table 3), propranolol and suxamethonium slowed the heart (Table 3), propranolol and phentolamine sometimes reduced shivering (Fig. 2) and the catecholamines increased blood pressure and frequently increased rectal temperature and activity (Fig. 7).

In anticipation of difficulties such as these, several approaches were

adopted. The use of sympatholytic agents and of catecholamines was based on the assumption that non-shivering thermogenesis is sympathetically mediated in the lamb as in other species studied (see Hull, 1966; Moore, 1966). Hexamethonium was abandoned early because of its marked circulatory effects (see also Dawes & Mestyán, 1963; Brück & Wünnenberg, 1965*b*). Alpha- as well as β-agents were used since there is little evidence about the effect of α-agents in the new-born. Beta-agents were used by Brück & Wünnenberg (1965*b*) and Heim & Hull (1966) in new-born guinea-pigs and rabbits, and they presented some evidence that the depression in oxygen consumption in the cold was not due to cardiovascular side effects. However, results with phentolamine have been unpredictable (Brück & Wünnenberg, 1965*b*) and failure of other sympatholytic drugs (pronethalol and phenoxybenzamine) to suppress non-shivering thermogenesis or catecholamine induced thermogenesis has been reported (Scopes & Tizard, 1963; Hull, 1964) in new-born kittens and rabbits.

The results of the three approaches, summarized in Table 7, provide a basis for estimating the contributions of shivering and non-shivering thermogenesis to summit metabolism in lambs of different ages, provided several assumptions are made. These are: that the fall in metabolism after phentolamine and propranolol was due to suppression of non-shivering thermogenesis alone; that the fall after muscular paralysis was due to suppression of shivering alone; that catecholamines stimulated resting oxygen consumption to the same extent as occurs during summit metabolism; and finally, that residual metabolism after simultaneous sympathetic blockade and muscular paralysis represents true basal metabolism (Fig. 6). Figure 8 and previous estimates of basal metabolism (Alexander, 1961*b*; Alexander & Williams, 1962) support the last assumption. Despite these assumptions, the data in Table 7 consistently indicate that, in new-born lambs, shivering is quantitatively at least as important as non-shivering metabolism, and that the non-shivering contribution declines rapidly with age, while that of shivering increases somewhat. They also indicate that the elaborate preparation of the animal did not affect summit metabolism; the mean value (3·5 l./kg.hr) obtained here was identical with that in earlier work (Alexander, 1962*a*). Although the precision of some of the estimates in Table 7 could have been affected by minor variations in procedure such as dose régime and previous treatment, the consistency of the results (Figs. 3–5 and 8; Table 6) leaves little doubt that the results in Table 7 are not artifacts resulting from these variations.

However, there is a significant inconsistency in the data. Sympathetic blockade appears to overestimate non-shivering thermogenesis in the older lambs, but not in the younger lambs (Table 7). This inconsistency

TABLE 7. Estimation of the contribution of shivering and non-shivering thermogenesis to summit metabolism, by use of sympatholytic agents, muscular paralysis, and thermogenic stimulation with catecholamines

	Estimated components of summit metabolism (l. oxygen/kg.hr)											
	Birth*						One month*					
Experimental agent	Shivering		Basal		Non-shivering†	Total	Shivering		Basal		Non-shivering†	Total
Phentolamine	—	2·7	—	—	0·8	3·5	—	2·1	—	—	0·6	2·7
Propranolol	—	2·3	—	—	1·2	3·5	—	1·9	—	—	0·8	2·7
Suxamethonium	1·9	—	—	1·6	—	3·5	2·0	—	—	0·7	—	2·7
Catecholamines	—	—	1·0	—	1·4	—	—	—	0·7	—	0·1	—
Combination of sympatholytic drug and suxamethonium	—	—	0·8	—	—	3·5	—	—	0·5	—	—	—‡
	(Calculated values—assuming values for basal and total (as indicated))											
Phentolamine	1·9		0·8§		0·8	3·5	1·6‖		0·5§		0·6‖	2·7
Propranolol	1·5		0·8§		1·2	3·5	1·4‖		0·5§		0·8‖	2·7
Suxamethonium	1·9		0·8§		0·8	3·5	2·0		0·5§		0·2	2·7
Catecholamines	1·3		0·8§		1·4	3·5‡	2·1		0·5§		0·1	2·7
Means	1·6		0·8		1·1	3·5	2·1		0·5		0·1	2·7
Percentage of total	46		23		31	100	78		18		4	100

* The appropriate values of oxygen consumption were calculated from the data in Figs. 3–6 and 8 using the regression coefficients shown in Table 6. Closely similar values were obtained by direct estimates from the figures.

† Response to cold as distinct from basal.

‡ Insufficient data.

§ Assumed values.

‖ Excluded from means (see text).

may result from cardiovascular effects of blockade which could be age dependent, or from interference with the supply of lipid for muscle metabolism (see Fritz, Davis, Holtrop & Dundee, 1958) in the older lambs, since sympathetic blockade will reduce mobilization of lipid from the white fat (see Wenke, 1966) that replaces the brown fat in the neonate (see below), and brown fat is believed to supply only small amounts of free fatty acid for metabolism elsewhere (Dawkins & Hull, 1964). Sympathetic blockade appeared to depress shivering in at least some of the younger lambs (Fig. 2), so that this approach appears to be quantitatively the least reliable although it has been the main approach of other workers.

The greater depression of oxygen consumption by β than by α sympatholytic agents is not easily explained. It could be due to differences in cardiovascular reactions or to differences in their mode or degree of inhibition of lipid mobilization (see Wenke, 1966); inhibition of adrenergic lipid mobilization by α-agents does not appear to be mediated by α-receptors, while inhibition by β-agents appears specifically related to their β-activity. In addition, weak lipid mobilizing properties have been ascribed to phenotolamine (Boshart, Smith, Will, Pirre, Perrine & Ringler, 1964) while propranolol is amongst the most effective β-inhibitors of lipid mobilization.

The data indicate that non-shivering thermogenesis in the lamb is sympathetically mediated, but they do not prove that the heat originates from brown adipose tissue. This is likely, however, because the macroscopic and histological appearance of the fat depots in near-term foetal and new-born lambs (Wensvoort, 1967, and G. Alexander, unpublished data) is consistent with the description of brown fat in other species (see Hull, 1966) and because the decline with age in the response of the lamb to catecholamines parallels the disappearance of brown fat (see below). The present work does not eliminate muscle and other tissues as sources of non-shivering thermogenesis, as reported to occur in the cold acclimated and functionally eviscerated curarized rat, in which exposure to cold or noradrenaline infusion increased oxygen consumption without muscular contraction (Jansky & Hart, 1963). However, the rapid rise in deep rectal temperature observed on exposure of new-born lambs to cold (Fig. 9 of Alexander, 1961*b*) is consistent with local heating by brown fat which is concentrated in the abdominal cavity of lambs; this rise may be analogous to that over the interscapular fat pad in the new-born rabbit exposed to cold (Dawkins & Hull, 1964).

The mean respiratory quotient of 0·90 (Table 2) during control measurements of summit metabolism suggests that carbohydrate plays an important role in summit metabolism, presumably via shivering, whereas under milder conditions the R.Q. indicates that fat is the main substrate for

thermogenesis in very young fasting lambs (Alexander, 1961*b*). However, the R.Q. is of doubtful significance since the periods of measurement were short and the lambs had not been deliberately fasted. The apparent changes after drug administration are also of doubtful significance, since they are confounded with progressive fasting and the periods of measurement were short.

In the three lambs examined, the response of non-shivering thermogenesis to adrenaline was similar to the response to noradrenaline. However, in subsequent studies (G. Alexander, unpublished) using lambs from another source, the metabolic response to adrenaline was usually less than that to noradrenaline and was sometimes absent. It is suggested that adrenaline stimulates brown adipose tissue, but at the same time may reduce the effectiveness of the stimulation by reducing blood flow through the tissue. In the guinea-pig, noradrenaline and adrenaline are equally effective (Dawes & Mestyán, 1963), whereas in the kitten (Moore & Underwood, 1960) and young rabbit (Dawes & Mestyán, 1963) adrenaline is relatively ineffective. These species differences could arise from effects of adrenaline on blood flow through brown adipose tissue.

In general, however, the present results add weight to the suggestion of Dawes & Mestyán that responsiveness to adrenaline is a feature of maturity at birth; and they indicate that non-shivering thermogenesis may be maximally stimulated by exogenous catecholamines (Table 7) as also suggested by the data of Hull & Segall (1965*b*).

The responsiveness to catecholamines steadily declined from birth (Fig. 8) and virtually disappeared after 3 weeks of age in lambs maintained under the mild conditions of the animal house and with increasing thermal insulation of the coat. A similar decline occurs in guinea-pigs (Brück & Wünnenberg, 1965*b*), in kittens and young rabbits (Scopes & Tizard, 1963), and in young rats (Moore & Simmonds, 1966); and is associated with the decline and virtual disappearance of brown fat (Brück & Wünnenberg 1965*c*; G. Alexander, unpublished data).

The experiments, so far, provide no objective evidence about the relative contributions of shivering and non-shivering thermogenesis in the lamb producing heat at rates much below maximum. The subjective observations (G. Alexander, 1962, and unpublished data) that heat production in many young lambs can be elevated without overt shivering, suggest that shivering may not occur until non-shivering thermogenesis is approaching its capacity. The R.Q. data support this suggestion (see above). Similar suggestions were made by Hull (1965) for the new-born rabbit and Brück & Wünnenberg (1966) for the new-born guinea-pig; and the experiments of the latter suggest that shivering begins when the temperature of the cervical vertebral canal falls owing to insufficiency of non-shivering thermogenesis.

Shivering in the new-born lamb thus appears quantitatively more important than in the new-born of the other species so far examined except possibly in the pig and ox, but the possibility remains that the thermogenic potential of shivering has not been adequately assessed in most species. Brück & Wünnenberg (1965*a*, *b*) assumed that oxygen consumption, and hence shivering, were near maximum when new-born guinea-pigs were exposed to the standard low temperature of 8° C, but apparently most, if not all of the animals maintained a normal colonic temperature. If shivering does not appear until the non-shivering thermogenic potential is approached, it is likely that the maximum oxygen consumption and thermogenic potential of shivering have been underestimated. The estimate of the contribution of shivering is also dependent on the assumption that shivering is not suppressed by pronethalol in the new-born guinea-pig as distinct from the older animals and that shivering thermogenesis is proportionately reflected by the electromyogram; the muscles could contribute a significant amount of heat by an increase in tone without overt shivering. Similarly, the evidence of Hull and colleagues that activity of muscle is of minor importance in the new-born rabbit is subjective and indirect; confirmation by more objective methods is required, particularly in young rabbits with depressed rectal temperatures.

Underestimation of the thermogenic potential of shivering could lead to erroneous conclusions in experiments where sympathetic drugs failed to depress cold induced thermogenesis; theoretically, shivering thermogenesis could almost exactly replace non-shivering thermogenesis.

Although shivering thermogenesis is very well developed in the new-born lamb, the possession of an additional source of heat is of clear survival advantage. Lambs are frequently exposed to cold, wet and windy weather at birth and the thermal insulation of the birth coat in most Merinos is low (Alexander, 1961*b*). Non-shivering thermogenesis greatly increases the range of climatic conditions in which homeothermy can be maintained. For example, it can be calculated from the results and earlier data (Alexander, 1962*a*) that, in the absence of non-shivering thermogenesis, a dry new-born lamb of 3·5 kg, with a fine birth coat, would become hypothermic in still air if the ambient temperature fell below −15° C; non-shivering, added to other sources of thermogenesis, would enable normal rectal temperatures to be maintained until air temperatures fell below −50° C. Similarly, it can be shown that no new-born lamb could maintain homeothermy, in the absence of non-shivering thermogenesis, at 20° C, in a wind of 15 km/hr and with water evaporating from a coat saturated with foetal fluids.

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